# Wingless signaling: **The inconvenient complexities of life**

Rachel T. Cox* and Mark Peifer*†

**Recent results suggest that our current model of Wingless/Wnt signal transduction is over-simplified.**

Addresses: *Curriculum in Genetics and Molecular Biology and †Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-3280, USA.

Correspondence: Mark Peifer
E-mail: peifer@unc.edu

Current Biology 1998, 8:R140–R144
http://biomednet.com/elecref/09609822008R0140



Nature is a home handywoman. Constrained by evolution, she does the job with the tools at hand, using a screwdriver for a hammer if necessary. This is well illustrated by the way particular cell signaling systems, such as that mediated by Wingless (Wg) in *Drosophila* and its Wnt homologs in vertebrates, direct diverse developmental decisions. The Wg/Wnt signaling system has been the subject of intense research in recent years, which has led to a snapshot of the machinery that transduces Wg/Wnt signals inside target cells. This machinery is neither elegant nor simple, but consists rather of a complex set of interacting proteins that were cobbled together by evolution. This is rather unfortunate for biologists, who as a rule prefer simple models, such as the current model of Wg/Wnt signaling which is illustrated in Figure 1 [1].

Wg/Wnt proteins are secreted ligands that interact with transmembrane receptors of the Frizzled (Fz) family. Signaling via the Fz receptor regulates levels of an effector protein known as Armadillo (Arm) in *Drosophila* and β-catenin (βcat) in vertebrates. In the absence of an extracellular signal, Arm/βcat is confined to cell–cell adhesive junctions; outside these it is rapidly destroyed by a multiprotein complex containing an kinase — Zeste-white 3 (Zw3) in *Drosophila* and glycogen synthase kinase (GSK) in vertebrates — and, in vertebrates at least, the product of the tumor suppressor gene *adenomatous polyposis coli* (*APC*). Wg/Wnt binding to Fz activates the downstream protein Dishevelled (Dsh), which somehow inactivates the destruction machinery, perhaps by inhibiting Zw3/GSK. This stabilizes non-junctional Arm/βcat; the levels of Arm/βcat consequently rise in the cytoplasm and nucleus, driving complex formation with DNA-binding transcription factors of the TCF/LEF family. The resulting complex binds to, and activates, Wg/Wnt responsive genes.

Models help to organize our thoughts and offer testable hypotheses. Of course, in constructing a model, some data may need to be hammered into place, and the inconvenient data that cannot be coaxed into place have to be left out. The models that are frequently illustrated in minireviews, and their attendant "minidogmas (and) mythinformation" thus cannot be viewed as the 'truth' [2], or they would narrow thought processes and squelch novel lines of research. We must be thoughtful iconoclasts, remembering that ultimately all models are wrong, fundamentally flawed or lacking the full complexity of systems shaped by evolution rather than intelligent design. We will thus use this forum to critique rather than prop up our model (Figure 1). It is increasingly clear that life is more complicated than portrayed there.

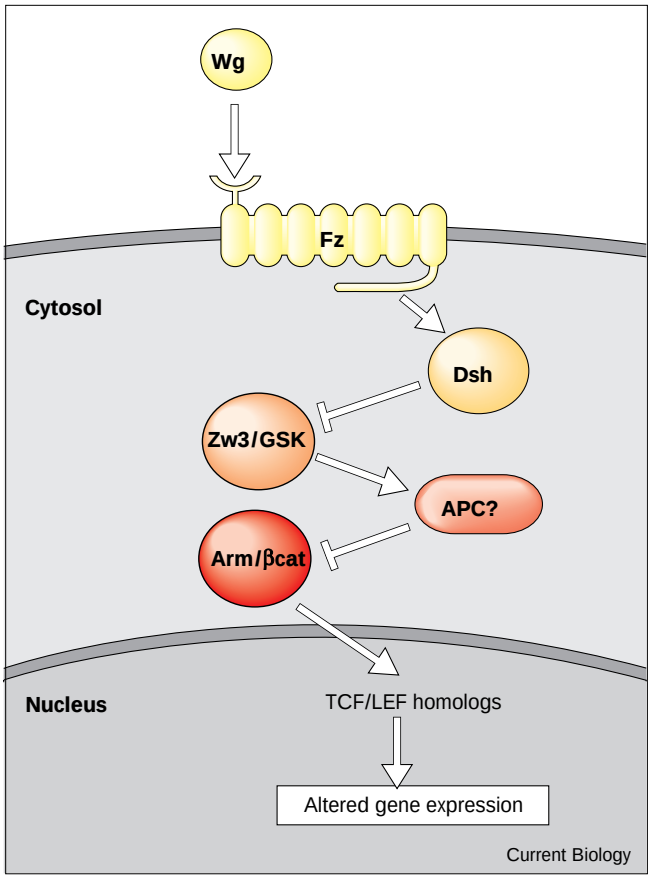
## Mixing and matching ligands and receptors

The demonstration that *Drosophila* Fz2 (DFz2) is a Wg receptor was a breakthrough in the field. The Wnt and Fz families are both large, however, raising the issue of which Wnt binds to which Fz. In experimental systems, ligands and receptors can be experimentally mixed and matched (reviewed in [3]), but what about during normal development? Genetic analysis in the nematode *Caenorhabditis elegans* provided the first evidence for a specific *in vivo* match between a Wnt ligand and Fz receptor. This work showed that the Wnt LIN-44 and the Fz LIN-17 together direct cell polarity during the post-embryonic development of *C. elegans*.

A more recent example concerns the four-cell stage of embryogenesis in *C. elegans*. Each of these four cells has a well-defined fate, with a distinct set of progeny cells whose lineages have been fully traced. Despite this apparently rigid relationship between cell lineage and developmental fate, cell–cell signaling plays an important part in *C. elegans* development. Signaling from the P2 cell polarizes her sister EMS, stimulating an asymmetric division that produces E, the endoderm progenitor, and MS, a mesoderm progenitor. Two groups [4,5] identified maternal effect *mom* ('*more mesoderm*') mutants in which this signaling event is disrupted, causing E to take on MS characteristics. *mom* mutants also affect mitotic spindle orientation. Cloning of the *mom-2* and *mom-5* genes identified a second matched ligand–receptor pair — *mom-2* encodes a Wnt, and *mom-5* encodes a Fz (Figure 2). As null *mom* alleles have variable penetrance, however, other Wnts and Fzs may play semi-redundant roles in this signaling process.

*Drosophila* Wg may act via multiple receptors [6]. Mutations altering different regions of the Wg protein independently affect two Wg functions in the ventral epidermis:

**Figure 1**




Insect and vertebrate Wg/Wnt signaling pathways share many homologous components. While many experiments support the view that the two pathways function in essentially identical ways, there are complications suggesting that this relatively simple, linear model is not complete.

the specification of naked cuticle and of denticle identity. This suggests that distinct functions of Wg are transduced through different Fz receptors. Wild-type Wg is thought to be transported through cells and released on the other side, thus propagating the signal from cell to cell. Disruption of signal propagation alters cell-fate specification, and different *wg* mutations differentially affect Wg translocation, suggesting that discrete classes of Wg receptor work in transport versus signal transduction.

### Bad hair days

Some Fz family receptors may use alternative signal transduction pathways to the one illustrated in Figure 1. In *Drosophila*, *fz* mutations disrupt tissue polarity, the compass by which epithelial cells tell direction. For example, wing hairs — actin-containing projections of single cells — always point distally in wild-type flies. In *fz* mutants, however, the wing hairs are disarrayed, pointing in random directions. Tissue polarity has been extensively examined, and numerous genes have been identified that act downstream of Fz (Figure 2). While Dsh is common to both the Wg and tissue-polarity pathways, some tissue polarity mutations do not affect Wg signaling. Fz itself, for example, is not essential for most Wg signaling; *fz* null mutations affect only tissue polarity. There are conflicting data concerning whether *wg* mutants affect tissue polarity; recent evidence suggests that Wg, Fz, Dsh and Zw3 together direct tissue polarity in *Drosophila* eyes [7].

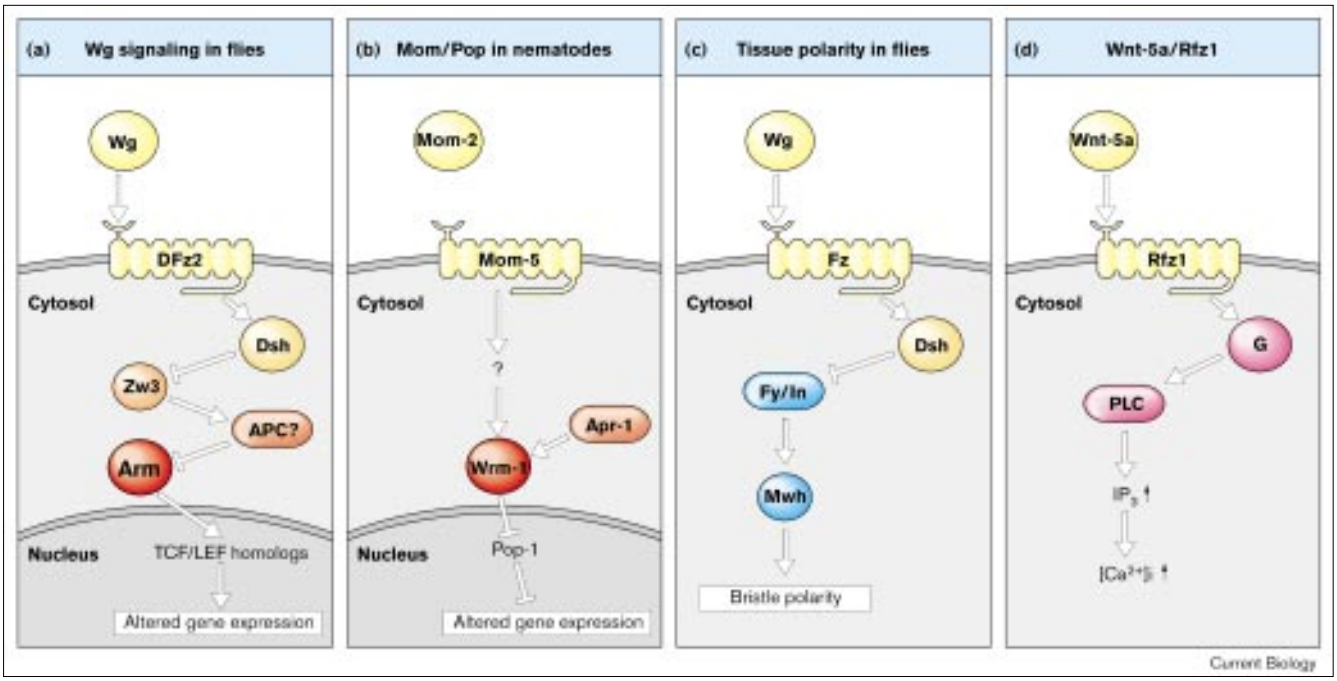
A gradient of Fz receptor activity across a field of cells is thought to orient the actin cytoskeleton. This model is supported by experiments in which an artificial gradient of Fz protein was generated across the developing wing [8]. Adler *et al.* [8] found that wing hairs pointed towards cells accumulating lower amounts of Fz protein, suggesting that cells orient themselves along a gradient of Fz activity. Perhaps Fz receptors are activated to different levels on the proximal and distal sides of single cells, creating polarity and directing the site of prehair initiation. It remains to be seen whether the activity gradient is formed by a diffusible Wnt ligand present in a concentration gradient along the proximal–distal axis, a gradient of Fz protein, or some other mechanism. It is intriguing that Wnt signaling in *C. elegans* embryos also polarizes cells.

Other Fz family members may use a third signaling pathway. Fz proteins are seven-pass transmembrane proteins, structurally analogous to G-protein-coupled receptors. In an interesting experiment involving extensive cross-species mixing and matching, Slusarski *et al.* [9] found that, after injection into zebrafish embryos, *Xenopus* Wnt-5a can act through rat Fz-2 to stimulate intracellular $Ca^{2+}$ release via the phosphatidylinositol pathway [9] (Figure 2). It is known that G-protein-coupled receptors can stimulate the phosphatidylinositol pathway, and, indeed, co-injection of pertussis toxin or a non-hydrolyzable GDP analog to inhibit G-protein action abolished the effect, supporting the view that heterotrimeric G-proteins are involved in the Fz2 pathway. There is evidence that the small GTPase RhoA acts downstream of the Fz receptor in the tissue-polarity pathway in *Drosophila*. Thus, *rhoA* mutants have tissue polarity defects similar to those of *fz* mutants [10], and the effects of overexpressing Fz in the *Drosophila* eye are blocked by *rhoA* mutations.

### Search and destroy

According to the standard model of Wg/Wnt signaling outlined above, APC and Zw3/GSK regulate the levels of non-junctional Arm/βcat. This view is supported by the elevated levels of free Arm protein in *zw3 Drosophila* mutants and of free βcat in mammalian cells expressing a truncated APC protein. There are problems with this model, however. The role of APC is more complex than the model suggests. The most damning evidence comes from *C. elegans*. Disrupting the function of a *C. elegans*

**Figure 2**




Different Wnt/Fz pairs appear to use quite distinct signal transduction pathways; four such pathways are illustrated. **(a)** The canonical pathway defined for *Drosophila* Wg, as in Figure 1. **(b)** The Mom/Pop pathway, which mediates endoderm induction in *C. elegans*. This pathway shares many proteins with the Wg pathway; thus, for example, Apr-1 is an APC relative, Wrm-1 an Arm relative, and Pop-1 a dTCF relative. In the case of APC/Apr-1 and dTCF/Pop-1, however, the data suggest that a protein that is required for signaling in one pathway antagonizes signaling in the other. **(c)** The third pathway mediates wing hair polarity in flies. This pathway uses the first Fz receptor to be identified; its ligand remains unknown. While the Wg and Fz pathways both transduce signal through Dsh, the more downstream components in the two pathways appear to be distinct (Fy, Fuzzy; In, Inturned; Mwh, Multiple wing hair). **(d)** The pathway triggered by XWnt-5a binding to Rfz1 in zebrafish embryos. This leads to an increase in intracellular $Ca^{2+}$ via a heterotrimeric G protein and the phosphoinositol pathway (PLC, phospholipase C; $IP_3$, inositol 1,4,5-trisphosphate).

APC homolog — achieved using a new RNA-inhibition technique — blocks Wnt signaling during endoderm induction [4] (Figure 2). This suggests that APC has a positive role in Wnt signaling, a view supported by the finding that expression of full-length APC in *Xenopus* activates Wnt signaling and causes axis duplication (an effect that requires βcat) [11]. How can this evidence that APC is a positive Wg/Wnt effector be reconciled with the results in tissue culture and tumors suggesting that APC is a negative regulator?

*APC* was first identified as a tumor suppressor gene in colon tumors, but the tumor cells are not *APC* null mutants — they contain truncated APC protein. The truncated APC protein binds βcat, but cannot downregulate βcat levels. APC may normally act both positively and negatively in the Wg/Wnt pathway, and perhaps truncated APC retains ability to promote signaling. Regulating signaling may be only one of APC's functions; there is evidence that APC may promote cell migration via an effect on microtubule stability, and βcat may antagonize this. Transfected cells expressing an indestructible mutant form of βcat make stable APC–βcat complexes and are dramatically altered in their ability to migrate, send out cellular processes, and associate with one another [12,13]. Thus, APC and βcat may regulate one another. Another potential component of the destruction complex is Axin, which negatively regulates Wnt signaling in mice [14]. Axin contains a 'regulator of G-protein signaling' (RGS) domain, which is intriguing given the potential G-protein coupling of certain Fz receptors.

Zw3/GSK regulates Arm/βcat stability, but the identity of its substrate remains controversial. The amino terminus of Arm/βcat is required for its destruction; if the putative phosphorylation sites in this region are mutated, Arm/βcat is no longer destroyed. These serines may be phosphorylated by Zw3/GSK; indeed, the serines occur within consensus GSK phosphorylation sites, less phosphorylated Arm accumulates in *zw3* mutants, and GSK phosphorylates βcat *in vitro* (see [15], for example). Zw3/GSK's role may not be this simple, however; *zw3* mutations may affect Arm phosphorylation indirectly, via an effect on stability, and other kinases clearly can phosphorylate Arm [16].

Perhaps Zw3/GSK's true target is APC — it has been shown that APC can be phosphorylated by GSK, promoting binding of APC to βcat, and that stabilization of the APC–βcat complex elevates APC phosphorylation [17].

Which kinase is it then that phosphorylates the amino terminus of Arm/βcat? The phosphorylation sites weakly resemble sites in IκB that are phosphorylated by the recently identified IκB kinases. IκB phosphorylation is thought to trigger ubiquitination and destruction, and βcat stability is also regulated by ubiquitination [18]. The current model lacks a connection to the ubiquitination machinery. F-box proteins, which target cell-cycle regulators for destruction, may provide a paradigm; by analogy, the APC–Zw3/GSK complex might include proteins that donate ubiquitin to Arm/βcat.

### Do all roads lead to the nucleus?

The Wg/Wnt signal thus stabilizes Arm/βcat, allowing it to accumulate and form a complex with DNA-binding proteins of the TCF/LEF family. This complex is thought to bind sites within promotors of Wg/Wnt responsive genes, activating their expression (reviewed in [1]). Consistent with this idea, *dTCF* mutations disrupt Wg signaling in *Drosophila*, mutant TCF proteins lacking the amino-terminal Arm/βcat-binding site antagonize Wg/Wnt signaling in *Drosophila* and *Xenopus*, and TCF/LEF binding sites in the promotors of Wg/Wnt responsive genes are required for their transcriptional activation.

Other data conflict with this model, however. Consider for example the Wnt signaling pathway involved in endoderm specification in *C. elegans*. Identified components of this pathway include a Wnt, an Arm homolog, and a TCF/LEF homolog (known as POP-1). Unlike *Drosophila*, where *wg*, *arm* and *dTCF* mutations cause similar mutant phenotypes, in *C. elegans*, the *mom* mutations of the Wnt and Arm homologs cause a phenotype opposite to that caused by the *pop-1* mutation of the TCF homolog (Figure 2). In *C. elegans*, therefore, Wnt signaling antagonizes POP-1 action [4,5].

The model suggests that Arm/βcat acts in the nucleus, but there are problems with this too. To test the idea, mutant forms of both βcat and its paralog, which were engineered to be tethered to the plasma membrane, were expressed in *Xenopus* [19,20]. The model predicts that these proteins should be inactive in signaling, as they are not expected to be able to move into the nucleus, yet they were both found to activate the Wnt pathway. Merriam *et al.* [19] suggest that TCF/LEFs normally repress Wg/Wnt-responsive genes, and that βcat antagonizes this by sequestering TCF/LEF. Some other observations are consistent with this view: TCF/LEF proteins may repress the Wnt responsive gene *siamois* [21], certain TCF/LEFs are inactive or act as repressors upon misexpression in *Xenopus*, and TCF/LEFs unable to bind Arm/βcat repress Wg/Wnt responsive genes. Miller and Moon [20], however, have reported data suggesting that tethered plakoglobin and βcat bind, and thus block, the destruction machinery, allowing endogenous wild-type βcat to accumulate and signal. Resolution of this requires removing endogenous βcat.

A final gap in the model concerns how Arm/βcat enters nuclei, as it lacks a nuclear localization signal (NLS). Increased levels of TCF/LEF promote βcat accumulation in nuclei [22], consistent with the view that TCF/LEF shepherds Arm/βcat into the nucleus. However, Arm mutants that cannot bind dTCF — at least in the yeast two-hybrid assay — still localize to nuclei *in vivo* [23], suggesting that there is a TCF-independent entry mechanism. Importin, the NLS receptor, contains repeat sequences similar to those found in Arm/βcat. This prompted examination of βcat's ability to mediate nuclear import — surprisingly, βcat can bind to nuclear pore proteins and be imported into nuclei *in vitro* without need for the NLS receptor [24].

The model ends with the TCF/LEF–Arm complex acting in the nucleus to turn on target genes. The target genes, however, are expressed in patterns that are subsets of domains of Wg signaling, suggesting that transcriptional enhancers integrate input from multiple signaling pathways. The midgut enhancer of *Drosophila Ultrabithorax*, for example, has response elements for both Wg and Dpp; expression requires both inputs [25]. Similarly, the *Drosophila* EGF receptor and Wg pathways may collide at the level of target gene promotors [26,27].

In the midst of complexity, one fact emerges: our model is wrong! Where does this leave us? We're left excited, driven to go back to the fly bench, the injection apparatus and the cold room to take the next snapshot in our continuing quest to understand the haphazard yet beautiful strategies that evolution has crafted to build the bodies of the beasts we study.

### References


1. Cavallo R, Rubenstein D, Peifer M: Armadillo and dTCF: a marriage made in the nucleus. *Curr Opin Genet Dev* 1997, 7:459-466.
2. Klymkowsky MW: Minireviews, minidogmas and mythinformation. *Bioessays* 1997, 19:537-539.
3. Moon RT, Brown JD, Yang-Snyder JA, Miller JR: Structurally related receptors and antagonists compete for secreted Wnt ligands. *Cell* 1997, 88:725-728.
4. Rocheleau CE, Downs WD, Lin R, Wittmann C, Bei Y, Cha Y-H, Ali M, Preiss JR, Mello CC: Wnt signaling and an APC-related gene specify endoderm in early ***C. elegans*** embryos. *Cell* 1997, 90:707-716.
5. Thorpe CJ, Schlesinger A, Carter JC, Bowerman B: Wnt signaling polarizes an early ***C. elegans*** blastomere to distinguish endoderm from mesoderm. *Cell* 1997, 90:695-705.
6. Hays R, Gibori GB, Bejsovec A: Wingless signaling generates pattern through two distinct mechanisms. *Development* 1997, 124:3727-3736.

7. Tomlinson A, Strapps WR, Heemskerk J: Linking Frizzled and Wnt signaling in ***Drosophila*** development. *Development* 1997, 124:4515-4521.
8. Adler PN, Krasnow RE, Liu J: Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels. *Curr Biol* 1997, 7:940-949.
9. Slusarski DC, Corces VC, Moon RT: Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. *Nature* 1997, 390:410-413.
10. Strutt DI, Weber U, Mlodzik M: The role of RhoA in tissue polarity and Frizzled signalling. *Nature* 1997, 387:292-295.
11. Vleminckx K, Wong E, Guger K, Rubinfeld B, Polakis P, Gumbiner BM: Adenomatous polyposis tumor suppressor protein has signaling activity in ***Xenopus*** embryos resulting in the induction of an ectopic dorsoanterior axis. *J Cell Biol* 1997, 136:411-420.
12. Barth AIM, Pollack AL, Altschuler Y, Mostov KE, Nelson WJ: Amino-terminal deletion of β-catenin results in stable co-localization of mutant β-catenin with APC protein and altered MDCK cell adhesion. *J Cell Biol* 1997, 136:693-706.
13. Pollack AL, Barth AIM, Altschuler Y, Nelson WJ, Mostov KE: Dynamics of β-catenin interactions with APC protein regulate epithelial tubulogenesis. *J Cell Biol* 1997, 137:1651-1662.
14. Zeng L, Fagatto F, Zhang T, Hsu W, Vasicek TJ, Perry WL, Lee JJ, Tilghman SM, Gumbiner BM, Constantini F: The mouse Fused locus encodes axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* 1997, 90:181-192.
15. Yost C, Torres M, Miller JR, Huang E, Kimelman D, Moon RT: The axis-inducing activity, stability, and subcellular distribution of β-catenin is regulated in ***Xenopus*** embryos by glycogen synthase kinase. *Genes Dev* 1996, 10:1443-1454.
16. Pai L-M, Orsulic S, Bejsovec A, Peifer M: Negative regulation of Armadillo, a Wingless effector in ***Drosophila***. *Development* 1997, 124:2255-2266.
17. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P: Binding of GSK-β to the APC/β-catenin complex and regulation of complex assembly. *Science* 1996, 272:1023-1026.
18. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R: β-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J* 1997, 16:3797-3804.
19. Merriam JM, Rubenstein AB, Klymkowsky MW: Cytoplasmically anchored plakoglobin induces a Wnt-like phenotype in ***Xenopus.*** *Dev Biol* 1997, 185:67-81.
20. Miller JR, Moon RT: Analysis of the signaling activities of localization mutants of β-catenin during axis specification in ***Xenopus***. *J Cell Biol* 1997, 139:229-243.
21. Brannon M, Gomperts M, Sumoy L, Moon RT, Kimelman D: A beta-catenin/XTcf-3 complex binds to the siamois promoter to regulate dorsal axis specification in ***Xenopus***. *Genes Dev* 1997, 11:2359-2370.
22. Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG, Kemler R: Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mech Dev* 1996, 59:3-10.
23. Orsulic S, Peifer M: An ***in vivo*** structure-function analysis of armadillo, the β-catenin homologue, reveals both separate and overlapping regions of the protein required for cell adhesion and wingless signaling. *J Cell Biol* 1996, 134:1283-1301
24. Fagotto F, Glück U, Gumbiner BM: Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of β-catenin. *Curr Biol* 1998, 8:181-190.
25. Riese J, Yu X, Munnerlyn A, Eresh S, Hsu S-C, Grosschedl R, Bienz M: LEF-1, a nuclear factor coordinating signalling inputs from ***wingless*** and ***decapentaplegic.*** *Cell* 1997, 88:777-787.
26. Szüts D, Freeman M, Bienz M: Antagonism between EGFR and Wingless signaling in the larval cuticle of ***Drosophila***. *Development* 1997, 124:3209-3219.
27. O'Keefe L, Dougan ST, Gabay L, Raz E, Shilo B-Z, DiNardo S: Spitz and Wingless, emanating from distinct borders, cooperate to establish cell fate across the Engrailed domain in the ***Drosophila*** epidermis. *Development* 1997, 124:4837-4845.